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Age-Related Ultrasound Changes in Muscle Quantity and Quality in Women

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1 **Abstract**

2 This study investigated the age-related changes in muscle quantity and quality in the trunk and limbs in women. A total of
3 128 females were classified into 4 age groups: young, middle-aged, young-old and old-old. Muscle thickness (MT) and
4 echo intensity (EI) were measured for the biceps brachii, quadriceps femoris, rectus abdominis, external oblique, internal
5 oblique and transversus abdominis, using B-mode ultrasonography. The EIs of the biceps brachii, quadriceps femoris and
6 transversus abdominis were significantly higher in the middle-aged group than in the young group; however, there were no
7 significant differences in MT. Compared with the young group, all other groups had significant changes in both the MT
8 and EI of the rectus abdominis, external oblique and internal oblique muscles. Thus, qualitative changes in muscle may
9 occur earlier than quantitative changes, and loss of muscle mass in the superficial abdominal muscles may occur earlier
10 than those in the other muscles.

11 *Keywords:* muscle quantity, muscle quality, ultrasonography, trunk muscles

12

13

1 Introduction

2 Sarcopenia, defined as age-related loss of skeletal muscle mass, strength, and function (Cruz-Jentoft et al. 2010), is
3 considered as a primary reason of loss of independence and falls (Wolfson et al. 1995). Previous studies have typically
4 assessed age-related loss of muscle mass through muscle volume or cross-sectional area using magnetic resonance imaging
5 (Janssen et al. 2000), or computed tomography (Overend et al. 1992), and muscle thickness (MT) using ultrasound
6 (Candow and Chilibeck 2005; Arts et al. 2007). However, actual loss of contractile muscle tissue with aging is greater than
7 the decrease in muscle size assessed using these standard imaging methods because they are affected by changes in muscle
8 quality or composition including fat, connective tissue and extracellular water content (Yamada et al. 2010; Fukumoto et
9 al. 2012b).

10 Recently, it has become possible to assess muscle quality in vivo by non-invasive ultrasound imaging in which an
11 enhanced echo intensity (EI) represents augmented fat and connective tissue within muscle (Pillen et al. 2009). Previous
12 studies by others and us have assessed MT and EI as indices of muscle quantity and quality in the limbs; these revealed
13 that decreases in MT and increases in EI occurred with aging (Arts et al. 2010; Fukumoto et al. 2012b). Thus, quantitative
14 and qualitative changes are known to occur in the limb muscles with age. However, no study has investigated the correlation
15 between the trunk and limb muscle changes with aging. Therefore, this study aimed to investigate the age-related changes
16 in muscle quantity and quality in the upper and lower limbs and trunk muscles in women.

17

18 Materials and Methods

19 A total of 128 women participated in this study after providing written informed consent. Of these, 28 were young (age

1 19–30 years) and 100 were community-dwelling, middle-aged and elderly women living independently (age, 53–92 years).
2 The middle-aged and elderly participants were divided into 3 categories: middle-aged (<65 years), young-old (65–74 years)
3 and old-old (≥ 75 years). The eligibility criteria were as follows: able to walk without assistive devices and climb stairs, no
4 dementia, no history of trauma, surgery, neuromuscular disorders, metabolic disorders, or acute and chronic diseases that
5 impair the muscle quantity, quality and strength, or physical function. The history of parturition (yes or no) was recorded
6 using a questionnaire. The ethics committee of Kyoto University Graduate School and the Faculty of Medicine approved
7 the study protocol.

8 Ultrasound examinations were performed using a B-mode ultrasound device (LOGIQ e; GE Healthcare UK Ltd.,
9 Chalfont, Buckinghamshire, UK) with an 8 MHz linear-array probe. Equipment settings were consistently set to the
10 following: gain (58 dB), dynamic range (69 Hz) and time gain compensation in the neutral position. Transverse ultrasound
11 images of 6 muscles were obtained on the right side as previously described (Arts et al. 2010; Fukumoto et al. 2012a) from
12 the biceps brachii (including the underlying brachialis), quadriceps femoris, rectus abdominis, external oblique, internal
13 oblique and transversus abdominis muscles. The measurement site for each muscle are shown in **Figure 1**. During
14 measurements, the participants were asked to lay in the supine position with their arms and legs extended and their muscles
15 completely relaxed. All examinations were performed once for each muscle with great care by the same experienced
16 investigator. Representative ultrasound images are shown in **Figure 2**.

17 The frozen ultrasound images were stored and numbered to blind the investigator regarding the subject's age for
18 further analysis. MT was assessed as an index of muscle quantity using an electric caliper on the ultrasound images. EI was
19 assessed as an index of muscle quality by computer-assisted 8-bit greyscale analysis using the standard histogram function

1 in Adobe Photoshop Elements (Adobe Systems Inc., San Jose, California, USA). The method and its reliability have been
2 described in more detail in other studies (Arts et al. 2010; Fukumoto et al. 2012a; Ota et al. 2012).

3 Results are presented as the mean \pm standard deviation. Group differences were analyzed using one-way analysis of
4 variance followed by Tukey's post hoc test. All analyses were performed using IBM SPSS 20.0 for Windows (IBM Corp.,
5 Armonk, New York, USA). P values of <0.05 were considered statistically significant.

6

7 **Results**

8 Table 1 summarises the physical characteristics of the participants. Compared with the young group, BMI was
9 significantly higher in the middle-age and the young-old groups ($p < 0.05$), but not in the old-old group. Table 2 summarises
10 the results of the ultrasound measurements. There were significant main effects in MT and EI for all muscles measured (p
11 < 0.05).

12 MT of the biceps brachii showed a significant difference only between the middle-aged and old-old groups ($p <$
13 0.05). MT of the quadriceps femoris was significantly thinner in the young-old and old-old groups than in the young group,
14 and that was significantly thinner in the old-old group than the middle-aged group ($p < 0.01$). Compared with the young
15 group, MT of the rectus abdominis, external oblique and internal oblique were significantly thinner in the other groups (p
16 < 0.01). MT in the transversus abdominis showed a significant difference only between the young and the young-old groups
17 ($p < 0.01$).

18 Compared with the young group, EI was significantly higher in the muscles of all other groups ($p < 0.05$). EI was
19 significantly higher in the biceps brachii of the young-old and old-old groups and in the quadriceps femoris and external

1 oblique in the old-old group than in the middle-aged group ($p < 0.05$). No significant differences were observed in EI of
2 the other muscles among the middle-aged, young-old and old-old groups.

3

4 **Discussion**

5 Generally, age-related muscle atrophy is greater in the lower than the upper limbs (Janssen et al. 2000; Arts et al.
6 2007). Our results also demonstrated that the MT of the quadriceps femoris in young-old and old-old adults decreased
7 when compared with those of that in young adults, while the biceps brachii showed smaller age-related atrophy. In contrast
8 to our data, Candow and Chilibeck (2005) have reported that the MT of the elbow flexors and knee extensors in older men
9 (59–76 years) were significantly thinner than those of younger men (18–31 years). A possible explanation for this
10 discrepancy is that age-related muscle atrophy may be more marked in men than women (Janssen et al. 2000).

11 Previous studies have shown that EI of the biceps brachii and quadriceps femoris shows an age-related change (Arts
12 et al. 2010; Fukumoto et al. 2012b). Our study further examined this age-related change dividing the subjects into four age
13 groups, and clarified that the change in EI starts from the middle age, and that this change in these muscles occur prior to
14 the change in MT, when no significant decrease in MT is yet seen. Thus, age-related changes in muscle quality may occur
15 earlier than losses of muscle quantity during the life span. These results indicate that adipose and connective tissue within
16 this muscle had increased, resulting in a substantial decrease in contractile tissue even in middle age. Physiological evidence
17 suggests that increased fat and fibrous tissue within the muscle is associated with poor muscle strength (Fukumoto et al.
18 2012b). Therefore, interventions aimed at decreasing intramuscular fat and fibrous tissues may be needed from middle age
19 onward.

1 Previous studies have reported age-related losses of the abdominal muscles mass (Kanehisa et al. 2004; Ota et al.
2 2012). Notably, Ota et al. (2012) reported that MT of the superficial abdominal muscles, such as the rectus abdominis,
3 external oblique and internal oblique, was thinner among middle-aged women than younger women, whereas there was no
4 corresponding decrease in the MT of the deep abdominal muscles, such as the transversus abdominis. Although our study
5 is consistent with these results, we additionally revealed that the MT of the superficial abdominal muscles decreased from
6 middle age without finding differences in the MT of the biceps brachii or quadriceps femoris muscles between young and
7 middle-aged women. These results suggest that age-related atrophy of the superficial abdominal muscles may occur earlier
8 than that of the limb muscles in middle age. Furthermore, the EI was higher in all the trunk muscles of the middle-aged
9 group when compared with the young group. Thus, we revealed that both quantitative and qualitative changes occurred in
10 the superficial abdominal muscles of middle-aged women. Notably, although age-related atrophy was reported to be less
11 in the transversus abdominis (Ota et al. 2012), it was clarified that the actual contractile tissue in this muscle is decreased
12 by augmented fat and connective tissue. Impairment in the trunk muscles can result in a loss of stability to the lumbo-pelvic
13 region (Urquhart et al. 2005). These results suggest that intervention that improves the quantity and quality of trunk muscles
14 may be needed from middle age. However, given that the differences in MT and EI of the trunk muscles were generally
15 insignificant among the middle-aged, young-old and old-old groups, it is possible that the quantity and quality of these
16 muscles is maintained beyond middle age. These findings may be affected by the history of parturition. No women in the
17 young group had experienced parturition, whereas 77% of the total participants in the middle-aged and elderly groups had
18 experienced it. Further study is required to clarify the effects of parturition.

19 The current study is the first to analyze MT and EI of the trunk and limbs in women of different ages. Because

1 ultrasound imaging is a low-cost, easy-to-operate and safe method, it is suitable in the clinical setting to evaluate
2 quantitative and qualitative changes in various muscles with aging. Changes in the muscle quantity and quality have also
3 been reported in patients with lower-limb osteoarthritis (Fukumoto et al. 2012a), metabolic disorders (Koster and Schaap
4 2015), or following stroke (Lee et al. 2015). Further studies using ultrasound to measure MT and EI and clarify muscle
5 characteristics of various diseases are required in future.

6 The present study has several limitations. One limitation was that the sample size in our study was small. Notably,
7 we did not included women aged 30–40 years due to difficulty in recruitment, during which age-related increases in the EI
8 might occur in the superficial abdominal muscles. However, in future, we aim to include a large number of women of all
9 ages over 18, including 30–40. Another limitation of this study was its cross-sectional design and that we included only
10 women. Further longitudinal studies in both men and women of all ages are required to confirm our results. In addition, we
11 measured only the right side. This was feasible because we excluded subjects whose muscle characteristics were expected
12 to be asymmetrical. However, it has been demonstrated that there was asymmetry between the left and right sides in the
13 muscle thickness of biceps brachii (Arts et al. 2010). Therefore, the results of muscle quantity and quality, particularly for
14 biceps brachii, may have been affected depending on whether or not measurements were taken from the side of the
15 dominant hand.

16 In conclusion, age-related changes in muscle quality may occur at an earlier age than losses of muscle quantity. In
17 addition, loss of muscle mass in the superficial abdominal muscles, such as the rectus abdominis, external oblique and
18 internal oblique, may occur from an earlier age than the loss of muscle mass in the limb and the deep abdominal muscles.

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Table 1. Physical characteristics of the participants (n = 128)

	Young (n = 28)	Middle-aged (n = 25)	Young-old (n = 49)	Old-old (n = 26)
Age (years)	21.5 ± 2.6 (19; 30)	60.5 ± 3.1 (53; 64)	69.8 ± 2.8 (65; 74)	79.3 ± 4.3 (75; 92)
Height (cm)	161.2 ± 4.7 (153.0; 172.0)	154.5 ± 5.8 (140.1; 162.8) **	151.2 ± 5.1 (137.1; 160.9) **	150.0 ± 6.1 (140.9; 164.9) **†
Weight (kg)	54.6 ± 5.6 (45.0; 66.2)	54.6 ± 7.9 (44.1; 70.8)	52.0 ± 7.2 (37.0; 66.8)	48.6 ± 5.5 (39.0; 64.0) **
BMI (kg/m ²)	21.0 ± 1.8 (16.7; 24.1)	22.9 ± 2.9 (18.1; 29.9) *	22.7 ± 2.6 (17.0; 28.8) *	21.6 ± 1.7 (18.9; 26.3)
Parturition (yes/no), n (%)	0 (0)/28 (100)	21 (84)/4 (16)	40 (81.6)/9 (18.4)	16 (61.5)/10 (38.5)

Data presented as mean ± standard deviations (minimum; maximum). BMI; Body mass index

Significant difference compared with young (*p < 0.05, **p < 0.01)

Significant difference compared with middle-aged (†p < 0.05, ‡p < 0.01)

Table 2. Results of ultrasound measurements (n = 128)

	Young (n = 28)	Middle-aged (n = 25)	Young-old (n = 49)	Old-old (n = 26)
MT (cm)				
Biceps brachii	2.36 ± 0.27	2.46 ± 0.34	2.28 ± 0.31	2.21 ± 0.29 †
Quadriceps femoris	4.55 ± 0.53	4.12 ± 0.69	3.81 ± 0.56 **	3.45 ± 0.71 **‡
Rectus abdominis	1.09 ± 0.18	0.81 ± 0.18 **	0.76 ± 0.16 **	0.70 ± 0.17 **
External oblique	0.79 ± 0.14	0.67 ± 0.19 *	0.58 ± 0.14 **	0.52 ± 0.14 **‡
Internal oblique	1.13 ± 0.27	0.78 ± 0.21 **	0.79 ± 0.25 **	0.79 ± 0.18 **
Transversus abdominis	0.44 ± 0.14	0.37 ± 0.10	0.34 ± 0.11 **	0.39 ± 0.10
EI (0-255)				
Biceps brachii	86.6 ± 9.7	96.7 ± 14.6 *	106.3 ± 13.5 **†	108.7 ± 9.3 **‡
Quadriceps femoris	78.4 ± 5.2	99.9 ± 9.4 **	101.8 ± 9.3 **	109.7 ± 9.4 **‡§§
Rectus abdominis	62.0 ± 13.0	117.7 ± 17.7 **	127.4 ± 17.6 **	124.8 ± 17.5 **
External oblique	79.6 ± 9.3	115.7 ± 11.9 **	120.7 ± 12.6 **	125.7 ± 10.5 **†
Internal oblique	59.6 ± 9.8	96.5 ± 14.5 **	101.9 ± 14.3 **	104.3 ± 14.2 **
Transversus abdominis	46.0 ± 13.1	80.3 ± 13.3 **	86.4 ± 17.0 **	87.7 ± 15.3 **

Data presented as mean ± standard deviations. MT; Muscle thickness, EI; Echo intensity

Significant difference compared with young (*p < 0.05, **p < 0.01)

Significant difference compared with middle-aged (†p < 0.05, ‡p < 0.01)

Significant difference compared with young-old (§§p < 0.01)

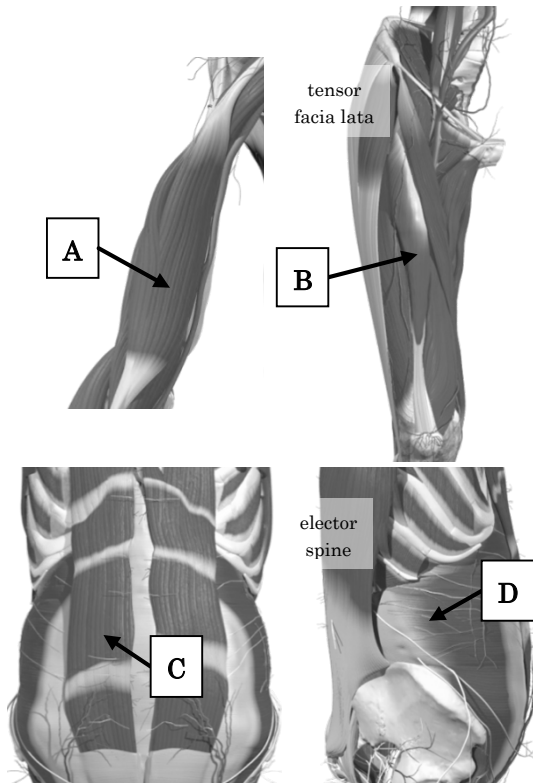


Fig 1. Ultrasound sites for each muscle. A: Biceps brachii, Two-thirds between the acromion and the antecubital crease. B: Quadriceps femoris, Midway between the anterior superior iliac spine and the proximal end of the patella. C: Rectus abdominis, 3 cm lateral to the umbilicus. D: External oblique, internal oblique and transversus abdominis, 2.5 cm anterior to the mid-axillary line, at the mid-point between the inferior rib and the iliac crest. (These figures were cited from Primal Pictures on OvidSP and Fukumoto et al. 2012a.)

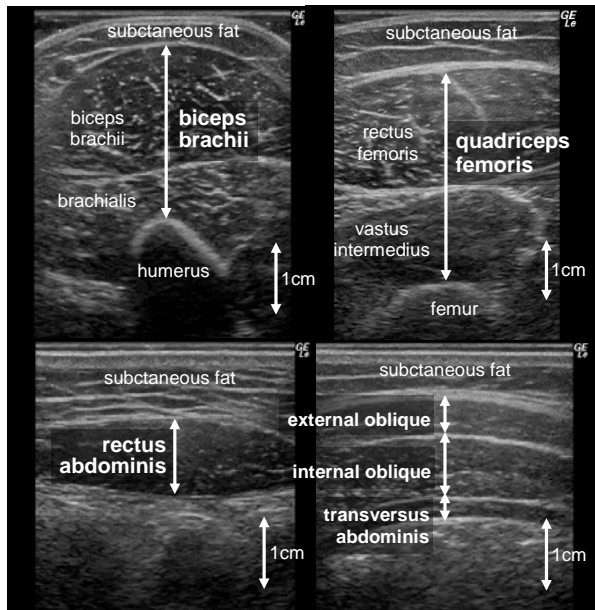


Fig 2. Representative ultrasound images.